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Genetic and genomic relationship between methane production measured in breath and fatty acid content in milk samples from Danish Holsteins

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Abstract. In this study the objective was to estimate the genetic and genomic relationship between methane-related traits and milk fatty acid profiles. This was done using two different estimation procedures: a single nucleotide polymorphism-based genomic relationship matrix and a classical pedigree-based relationship matrix. Data was generated on three Danish Holstein herds and a total of 339 cows were available for the study. Methane phenotypes were generated in milking robots during milking over a weekly period and the milk phenotypes were quantified from milk from one milking. Genetic and genomic parameters were estimated using a mixed linear model. Results showed that heritability estimates were comparable between models, but the standard error was lower for genomic heritabilities compared with genetic heritabilities. Genetic as well as genomic correlations were highly variable and had high standard errors, reflecting a similar pattern as for the heritability estimates with lower standard errors for the genomic correlations compared with the pedigree-based genetic correlations. Many of the correlations though had a magnitude that makes further studies on larger datasets worthwhile. The results indicate that genotypes are highly valuable in studies where limited number of phenotypes can be recorded. Also it shows that there is some significant genetic association between methane in the breath of the cow and milk fatty acids profiles.

Additional keywords: correlations, dairy cattle, fatty acids, heritability, methane.

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Introduction

Methane (CH_4) is a potent greenhouse gas that is ~25 as active as carbon dioxide. Dairy cows emit CH_4 while digesting and their contribution to the worldwide emission of greenhouse gas is substantial. Several mitigation strategies have been suggested: feeding, vaccination, management but also genetic selection (Cottle *et al.* 2011). In order to select for a given trait, the trait must show genetic or genomic variability and a substantial amount of data from individual cows are needed.

Several studies have worked on developing methods for large-scale phenotyping of individual CH_4 measurements (Garnsworthy *et al.* 2012; Lassen *et al.* 2012). These methods focus on CH_4 recordings while cows are being milked in automatic milking systems. However, even with this effort, it will still be very time consuming and difficult to make direct individual CH_4 measurements on all cows or the majority of cows in a population to make genetic selection possible. Therefore, indicators are needed, if CH_4 emission from dairy cattle should be decreased. It has been shown that feed intake can be used to predict CH_4 emission (de Haas *et al.* 2011). It is even harder to imagine that feed intake data rather than CH_4 data should be available on the majority of the cows in a population.

A stoichiometric relationship between CH_4 and ruminal acetate, propionate, and butyrate was proposed by Demeyer and Van Nevel (1975). These volatile fatty acids, which are primarily formed in the rumen act as precursors for the *de novo* synthesis of milk fatty acids in the mammary tissue. Thus, milk fatty acid data has been used to predict CH_4 emission in several studies (Dijkstra *et al.* 2011; Dehareng *et al.* 2012). Additionally, if one would like to implement CH_4 in the breeding goal it is important to know the correlation to other traits. Kandel *et al.* (2013) showed positive genetic correlations between milk mid-infrared spectre (MIR) CH_4 in g/day and energy-corrected milk (ECM), fat yield, and protein yield (Kandel *et al.* 2013). This means that a decrease of CH_4 should have negative impacts on milk, fat and protein yields. Due to the relative small datasets with CH_4 measures standard errors are usually high. Recently it was shown that single nucleotide polymorphism (SNP) information can be used to estimate the heritability in small datasets of ~400 animals (Krag *et al.* 2013*a*, 2013*b*).

This methodology has successfully been applied to fatty acid concentrations in milk fat with moderate estimates of heritability ranging from 0.10 for C18:1 *trans*-11 to 0.34 for C8:0 and C10:0 (Krag *et al.* 2013*b*). They showed that the SNP markers capture

the population structure well and that SNP markers could be used as an alternative to traditional pedigree-based methods. Use of the genomic relationship matrix will decrease the standard error of the estimates as the true genetic relationship between animals is utilised in a better way than when a traditional pedigree is applied.

The aim of this study was to estimate the genetic and genomic heritability for CH_4-related traits and fatty acid concentrations in milk fat as well as to estimate the genetic and genomic correlation between CH_4-related traits and fatty acids in milk. This is done on a limited dataset but can give an indication of how milk fatty acids and CH_4 emission relates genetically in dairy cattle.

Materials and methods

Data were generated on three commercial farms in Denmark. A total of 339 Holstein cows were available for this study. Several phenotypes were generated or extracted for the analysis (Table 1). Methane measuring equipment was installed in milking robots using a portable Fourier transformed infrared spectre measuring device making a registration of CH_4 and carbon dioxide every 5 s (Lassen *et al.* 2012). This data was merged with traffic data from each milking robot so that each cow got a phenotype for each visit in the robot. The equipment was installed in each milking robot for 7 days. From each visit, bodyweight and milk yield were also recorded by the milking robot. From these records a weekly mean of bodyweight and milk production was calculated. This information was used to estimate daily CH_4 production in grams per day (Madsen *et al.* 2010). Methane in g/day (CH4_GRAMS) is calculated based on heat-producing units (HPU), which is equal to ($5.6*\text{liveweight}^{0.75} + 22*\text{FPCM} + 1.6*10^{-5}*\text{days carried calf}$). For each HPU a cow produces 180 g of carbon dioxide per h. CH4_GRAMSw is thereby CH4_RATIO*180*24*HPU. Also CH_4 per kg of ECM (CH4_MILK) and the ratio between CH_4 and carbon dioxide CH4_RATIO were used as CH_4-related traits. Overall fat and protein percentages in milk were derived from the national milk recording system based on the nearest milking record from the week where CH_4 measurements were taken. Also milk samples were taken to measure milk fatty acid content in the milk. This was done by gas chromatography, essentially as described by Larsen *et al.* (2013).

The pedigree file contained 8049 animals and was traced back as far as possible in the national cattle database.

Table 1. Overall mean, standard deviation (s.d.), minimum (min.) and maximum (max.) values for the phenotypes used in the study
Values are in g/100 g fat

	Mean	s.d.	Min.	Max.
Energy-corrected milk	36.9	7.7	18.8	61.7
Weight	647.3	68.3	467	890
CH4_GRAMS	395	57.8	283	548
CH4_MILK	11.04	2.23	9.84	18.72
CH4_RATIO	0.072	0.01	0.054	0.11
C6:0	2.75	0.40	1.23	4.13
C8:0	1.44	0.27	0.34	2.40
C10:0	3.17	0.69	0.57	5.54
C12:0	3.68	0.89	0.74	6.21
C13:0	0.14	0.03	0.07	0.25
C14:0	11.58	1.95	4.06	15.32
C14:1	0.99	0.33	0.21	2.06
C15:0	1.10	0.24	0.50	1.87
C16:0	31.30	3.36	22.33	42.89
C16:1	1.78	0.49	0.90	4.12
C17:0	0.56	0.10	0.37	1.13
C18:0	9.53	1.99	5.99	17.70
C18:1*cis*9	20.31	3.97	13.19	34.70
C18:1*trans*11	1.34	0.29	0.83	2.21
C18:2n6*cis*	1.87	0.24	1.30	2.82
C18:3n3	0.53	0.087	0.31	0.85
CLA*cis*9, *trans*11	0.48	0.11	0.23	0.88
Fat (g/100 g milk)	3.93	0.79	1.92	7.03
Protein (g/100 g milk)	3.32	0.33	2.36	4.33

Genotyping

In total 339 DH cows were genotyped with the BovineSNP50 beadchip (http://www.illumina.com/Documents/products/datasheets/datasheet_bovine_snp5O.pdf, verified 18 November 2015). Genomic DNA was extracted from ear tissue. The platform used was an Illumina Infinium II Multisample assay device. SNP chips were scanned using iScan and analysed using Beadstudio software version 3.1. The quality parameters used for the selection of SNP in the GWAS were minimum call rates of 80% for individuals and 95% for loci. Marker loci with minor allele frequencies (MAF) below 1% were excluded. The quality of the markers was assessed using the GenCall data analysis software of Illumina. Individuals with average GenCall scores below 0.65 were excluded following Teo *et al.* (2007). The SNP positions were based on the *Bos taurus* genome assembly (*Btau_4.0*) (Liu *et al.* 2009). In total 39 121 SNP markers were used.

Calculation of the G-matrix

The calculation of the genomic relationship matrix has been described in detail by Buitenhuis *et al.* (2011). For each chromosome, a genomic relationship matrix as described by the first method presented in VanRaden (2008) was calculated as follows: Let **M** be a matrix with dimensions of the number of individuals (n) by the number of loci (m) that specifies which marker alleles each individual inherited. The elements of **M** were set to –1, 0, 1 for the homozygote, heterozygote and the other homozygote, respectively. The diagonals of **M**'**M** counts the number of homozygous loci for each individual and off diagonals measure the number of alleles shared by relatives. Let the frequency of the second allele at locus i be p_i, and let **P** contain the allele frequencies, such that column i of **P** equals $2(p_i - 0.5)$. Subtraction of **P** from **M** gives **Z**, which is needed to set the expected mean value to 0. The genomic relationship matrix **G** was then calculated as $\mathbf{ZZ}'/[2\sum p_i(1 - p_i)]$ (VanRaden 2008).

Statistical analyses

The data was analysed using equivalent models for the different traits but with two different types of relationship structures. First approach was a standard pedigree-based approach where the inverse of the animal model A matrix is set up. In the second approach a SNP-based genomic relationship matrix was set up.

The following linear animal model was used to infer genetic parameters for CH4_GRAMS.

$$y_{ij} = \mu + herd + month + robot * herd + lact_j + \beta * \dim + \beta * e^{-0.05} * \dim + a_i + e_i$$

Where y_{ijklm} is the dependent phenotype CH4_GRAMS, μ is the overall intercept, $herd_i$ is a fixed effect of the herd-id where cows were measured, $month_j$ is a fixed effect, *robot*herd* is the robot by herd interaction, $lact_k$ is a fixed effect of the lactation number at recording and *dimL* is the days in milk at recording. Days in milk was modelled with a linear regression and an exponential Wilmink term to take changes in early lactation into account. This fits a lactation curve to the data (Wilmink 1987). The β's are fixed regression coefficients. a_m is the random animal effects and e_{ijklm} is the random residual effect.

The following linear animal model was used to infer genetic parameters for CH4_MILK, ECM and weight.

$$y_{ij} = \mu + herd + month + lact_j + \beta * \dim + \beta * e^{-0.05} * \dim + a_i + e_i$$

Where y is the dependent phenotype CH4_MILK, ECM or weight, μ is the overall intercept, *herd* is the herd-id where cows were measured, *month* year* is the month by year interaction, *lact* is the lactation number at recording and *dim* is the days in milk at recording. Days in milk was modelled with a linear regression and a Wilmink term to take changes in early lactation into account. The β's are fixed regression coefficients; *a*'s are the random animal effects and *e* is the random residual effect.

The random effects for all models are assumed to be independently and normally distributed with means of zero. $\mathbf{G}_0$ is a matrix containing the additive genetic variance. **A** is a matrix with the additive genetic relationship of all animals. Pe_0 is a matrix containing the permanent environmental variance for CH4_RATIO and R_0 is a matrix with the residual variance. **I** is the identity matrix containing as many rows and columns as there are records for each trait.

$$\mathrm{Var}\begin{pmatrix} a \\ pe \\ e \end{pmatrix} \sim N\left(0; \begin{bmatrix} G_0(A & 0 & 0 \\ 0 & Pe_0(I & 0 \\ 0 & 0 & R_0(I \end{bmatrix}\right)$$

Variance and covariance components are estimated using the AI-REML procedure in DMU (Madsen and Jensen 2014). Correlations were estimated using a bi-variate model between CH4_RATIO, CH4_GRAMS, CH4_MILK, ECM, weight of all milk fatty acids and standard errors were estimated using a Taylor series approximation.

Results and discussion

Overall mean, standard deviation, minimum and maximum values for all the traits analysed are presented in Table 1. The milk data are generally in agreement with the values obtained in another study on Danish Holstein (Krag *et al.* 2013*b*). The average CH_4 production was 395 g per day and the CH_4 production in grams per kilo ECM was 11.04. This is in agreement with other studies on large-scale CH_4 recordings in dairy cattle (Lassen *et al.* 2012). Genetic and genomic heritabilities are shown in Table 2. For most milk fatty acids heritability estimates were moderate to low (0.00–0.6). For the three CH_4 traits the heritability was ~0.2 ranging from 0.09 to 0.26. Heritabilities estimated using either the SNP-based genomic relationship matrix or the pedigree-based relationship matrix are in agreement, but in most cases, standard error of the estimates are lower for the SNP-based genomic relationship matrix (mean standard error of 0.13) compared with the classical pedigree-based relationship matrix (mean standard error of 0.14) though not significant. Heritability estimates for C18 fatty acids are comparable to estimates found in Krag *et al*. (2013*a*), whereas the estimates for the *de novo* fatty acids are somewhat lower and estimates for C14:1 and C16:1 and to some extent C15:0 and C17:0 are higher. The difference in heritability estimates in the study by Krag *et al*. (2013*a*) could be due to a more stringent selection of cows, where daughter groups of limited size were achieved, whereas in this study, all animals were analysed without any restrictions on relationship. However, data recording was restricted to three farms, which means a small number of sires were used. This means that the cows in the current dataset might be more related compared with the cows in the Krag *et al*. (2013*a*) dataset.

Table 2. Pedigree-based and genomic heritabilities with standard errors

	H^2 pedigree	H^2 genomic
CH4_GRAMS	0.25 ± 0.16	0.24 ± 0.15
CH4_MILK	0.20 ± 0.16	0.26 ± 0.14
CH4_RATIO	0.16 ± 0.15	0.09 ± 0.11
C6:0	0.22 ± 0.15	0.12 ± 0.11
C8:0	0.17 ± 0.13	0.12 ± 0.11
C10:0	0.10 ± 0.11	0.09 ± 0.11
C12:0	0.11 ± 0.11	0.13 ± 0.12
C13:0	0.20 ± 0.16	0.19 ± 0.15
C14:0	0.16 ± 0.17	0.08 ± 0.10
C14:1	0.44 ± 0.18	0.60 ± 0.19
C15:0	0.36 ± 0.19	0.60 ± 0.21
C16:0	0.21 ± 0.13	0.17 ± 0.12
C16:1	0.20 ± 0.15	0.51 ± 0.19
C17:0	0.15 ± 0.13	0.23 ± 0.14
C18:0	0.11 ± 0. 12	0.17 ± 0.13
C18:1*cis*9	0.07 ± 0.10	0.02 ± 0.09
C18:1*trans*11	0.06 ± 0.11	0.10 ± 0.10
C18:2n6*cis*	0.15 ± 0.12	0.12 ± 0.11
C18:3n3	0.03 ± 0.11	0.00 ± 0.10
CLA*cis* 9, *trans*11	0.13 ± 0.12	0.18 ± 0.12
Fat (g/100 g milk)	0.19 ± 0.16	0.21 ± 0.14
Protein (g/100 g milk)	0.24 ± 0.14	0.22 ± 0.13
Mean s.e.	0.14	0.13

High heritabilities for C14:1 and C16:1 suggest that the desaturase activity underlying these fatty acids, to a large extent, is genetically regulated, which might be related to variation within the stearoyl-CoA desaturase 1 gene. However, in both studies the fatty acid heritability estimates are hardly significant due to relatively high standard errors. The differences in heritabilities for C14:1 and C16:1 between the pedigree-based and the SNP-based analysis is though probably largely due to randomness. In another study of 1800 Dutch Holstein cows intra-herd heritabilities were estimated for 14 milk fatty acids

and estimates were somewhat higher than in this study ranging from 0.24 for C18:0 to 0.72 for C10:0 (Bouwman *et al.* 2011). In a UK study of 2408 Holstein cows heritability estimates for 16 milk fatty acids ranged from 0.00 for C18:1, *trans*-11 to 0.28 for C14:1, *cis*-9 (Garnsworthy *et al.* 2010). The magnitude of the heritabilities found in the present study seems to be closer to those found by Garnsworthy *et al*. (2010) than those found by Bouwman *et al*. (2011). This can be due to the relaxed selection of animals based on the whole herd in the present study as well as in the study by Garnsworthy *et al*. (2010).

Genetic and genomic correlations were highly variable between methods and estimates are all over the parameter space (Table 3). Also in some cases, there was problem with obtaining convergence and thereby reasonable estimates. This was especially true for some of the C18 fatty acids where standard errors of the estimates sometimes were even higher than 1. As for the heritability estimates, the standard errors were higher for the pedigree-based estimates (mean standard error of 0.56, 0.61 and 0.66 for correlations with CH4_GRAMS, CH4_MILK and CH4_RATIO, respectively), compared with estimates using the SNP-based genomic relationship matrix (mean standard error of 0.44, 0.43 and 0.63 for correlations with, CH4_GRAMS, CH4_MILK and CH4_RATIO, respectively). The *de novo* fatty acids in particular seem to be significantly negatively correlated CH4_MILK, when using both methods, whereas C13:0, C15:0 and partly C17:0 had significantly positive genomic correlations with all CH_4 traits. The uneven saturated fatty acids are generated by the microbiota in the rumen and the content of these fatty acids in milk has previously been associated with the molar proportion of volatile fatty acids in the rumen (Vlaeminck *et al*. 2006). The standard errors of the estimates shown in this study are substantial, and it might not be possible to come to the same results on another similar study in another population of animals. SNP-based genomic relationship matrices to estimate heritability were shown to provide smaller standard error on the estimates compared with classical pedigree-based heritabilities. This is in line with the results of Krag *et al*. (2013*a*), who showed that when measures are hard or expensive to obtain reliable heritability estimates can be obtain on datasets containing ~400 animals with registrations and genotypes on traits with heritabilities higher than 0.15. In this study, we are right on the border of these thresholds for many of the traits and therefore also several heritabilities are not significantly different from 0. This is similarly the case for the genetic and the genomic correlations between the CH_4 traits and the milk component traits. There is a positive effect on the standard error of the estimates to use a SNP-based genomic relationship matrix rather than a classical pedigree-based relationship matrix. But again the data collected in this study is on the border of the data that is needed to provide significant genetic or genomic correlations even though the results show that the standard error decreases with use of DNA information. Therefore, it can be beneficial to use the resources on getting genotypes rather than getting more expensive phenotypes.

Using MIR to predict other phenotypes is indeed appealing. The phenotypic and genetic variability of CH_4 production (g/day) and CH_4 intensity (g/kg ECM) has been predicted by MIR. However, such estimates based on predicted CH_4 are heavily smoothed and are based on traits that already have high heritability, so they are expected to have high heritability themselves. In 2013, Kandel *et al*. estimated genetic parameters of MIR predicted CH_4 traits by using single trait random regressions test-day models from 679 444 test-day records collected from Holstein cows in their first three lactations. The calculated heritability values were ~0.10 for CH_4 in g/day (0.12, 0.10 and 0.09 for the 1st, 2nd, and 3rd parity, respectively). The

Table 3. Pedigree-based and genomic correlations with standard errors
NC, model did not converge

	Correlations pedigree			Correlations genomic		
	CH4_GRAMS	CH4_MILK	CH4_RATIO	CH4_GRAMS	CH4_MILK	CH4_RATIO
C6	–0.10 ± 0.49	–0.65 ± 0.49	–0.24 ± 0.59	–0.48 ± 0.55	–0.71 ± 0.45	–0.46 ± 0.68
C8	–0.09 ± 0.53	–0.80 ± 0.57	–0.08 ± 0.65	–0.06 ± 0.56	–0.60 ± 0.51	0.18 ± 0.74
C10	–0.10 ± 0.64	–0.82 ± 0.68	–0.18 ± 0.80	0.01 ± 0.61	–0.51 ± 0.59	0.38 ± 0.78
C12	0.066 ± 0.63	–0.63 ± 0.69	–0.06 ± 0.75	0.24 ± 0.49	–0.17 ± 0.51	0.58 ± 0.63
C13	–0.77 ± 0.37	–0.66 ± 0.98	0.56 ± 0.76	0.64 ± 0.49	0.57 ± 0.42	1.00 ± 0.68
C14	0.51 ± 0.81	0.09 ± 0.62	–0.39 ± 0.65	0.35 ± 0.54	0.13 ± 0.59	0.53 ± 0.71
C14_1		–0.09 ± 0.43	0.37 ± 0.42	0.38 ± 0.28	0.11 ± 0.28	0.29 ± 0.40
C15	–0.37 ± 0.37	0.40 ± 0.57	NC	0.87 ± 0.30	0.50 ± 0.29	1.00 ± 0.37
C16	–0.13 ± 0.48	–0.05 ± 0.53	0.04 ± 0.57	–0.25 ± 0.49	0.23 ± 0.45	–0.14 ± 0.64
C16_1	NC	NC	–	0.28 ± 0.36	0.11 ± 0.30	0.57 ± 0.60
C17	0.18 ± 0.54	0.39 ± 0.54	0.89 ± 0.64	0.62 ± 0.50	0.28 ± 0.36	1.00 ± 0.71
C18	–0.74 ± 0.65	0.18 ± 0.61	–0.02 ± 0.68	–0.25 ± 0.46	–0.06 ± 0.45	0.07 ± 0.69
C18:1*cis*9	0.25 ± 0.70	0.58 ± 0.79	0.06 ± 0.88	0.70 ± 2.44	–1.00 ± 1.95	0.11 ± 1.59
C18:1*trans*11	–0.87 ± 0.76	–0.70 ± 0.76	–0.71 ± 0.77	0.10 ± 0.61	0.03 ± 0.52	–0.27 ± 0.76
C18:2n6*cis*	0.42 ± 0.51	0.33 ± 0.66	0.43 ± 0.65	0.76 ± 0.45	0.22 ± 0.48	0.48 ± 0.62
C18:3n3	NC	NC	NC	1.00 ± 1.16	1.00 ± 1.97	1.00 ± 1.50
CLA*cis*9, *trans*11	0.33 ± 0.60	–0.19 ± 0.58	–0.09 ± 0.64	0.53 ± 0.46	–0.07 ± 0.42	0.13 ± 0.62
Fat	0.37 ± 0.49	0.59 ± 0.51	0.39 ± 0.58	–0.15 ± 0.48	0.11 ± 0.40	0.49 ± 0.57
Protein	0.77 ± 0.35	0.78 ± 0.31	0.85 ± 0.48	0.39 ± 0.42	0.46 ± 0.32	1.00 ± 0.61
Mean s.e.	0.56	0.61	0.66	0.44	0.43	0.63

heritability for CH_4 intensity was slightly higher with values ~0.15 (0.18, 0.12, and 0.14 for cows in their first three parities). These results suggest a relatively low heritability of CH_4 emission by dairy cows. Kandel *et al*. (2014) studied the consequences of selection for environmental impact traits in dairy cows using MIR data to predict CH_4 emission and setting up a selection index that include MIR-based CH_4 emission together with other traits of economic importance. These authors used CH_4 intensity (g/kg of milk) and calculated approximate genetic correlations from estimated breeding values. Negative approximate genetic correlations were observed between CH_4 intensity and milk yield (–0.67), fat yield (–0.13), protein yield (–0.46), longevity (–0.07), and average of conformation traits (–0.23). Positive approximate correlations were observed for fertility (0.31) and body condition score (0.27) in the study by Kandel *et al*. (2013). Based on these correlations and by putting a hypothetical 25% weight on CH_4 intensity on the current Walloon genetic evaluation index and proportional reduction on other selection traits, the response to selection was a reduction of CH_4 intensity by 24%, increase in milk yield by 30%, fat yield by 17%, protein yield by 29%, somatic cells score by –15%, longevity by 24%, fertility by –11%, body condition score by –13%, and conformation traits by 24%. These results suggest that a decrease of CH_4 intensity could have a negative impact on the cow fertility but a positive effect on the longevity but it needs to be tested on independent data as well as on data where more CH_4 observations are available.

Conclusion

Some milk fatty acids are genetically correlated with CH_4 emission in dairy cattle. In this study it was mainly C13:0, C15:0 and C17:0 that showed significant genetic and genomic correlations with CH_4 emission. Using a SNP-based genomic relationship matrix rather than pedigree-based relationship matrix provided estimates with lower standard errors. In small studies with limited sample size it is important to use animals that are genotyped to get more reliable estimates.

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